

# PATENT COOPERATION TREATY

LJz

18. MAY 2006/690

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

## PCT

### NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

To:

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Date of mailing  
(day/month/year)

16.05.2006

Applicant's or agent's file reference  
P2051PC00

#### IMPORTANT NOTIFICATION

International application No.  
PCT/DK2005/000126

International filing date (day/month/year)  
24.02.2005

Priority date (day/month/year)  
24.02.2004

Applicant  
CHR. HANSEN AS et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.
4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/B/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

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# PATENT COOPERATION TREATY



## PCT

### INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

LJz  
18 MAY 2006

Applicant's or agent's file reference P2051PC00	<b>FOR FURTHER ACTION</b>		See Form PCT/PEA/416
International application No. PCT/DK2005/000126	International filing date (day/month/year) 24.02.2005	Priority date (day/month/year) 24.02.2004	
International Patent Classification (IPC) or national classification and IPC INV. C12N1/00 C12N1/04			
Applicant CHR. HANSEN AS et al.			
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 4 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input checked="" type="checkbox"/> sent to the applicant and to the International Bureau a total of 3 sheets, as follows:</p> <p style="margin-left: 40px;"><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p>b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>			
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the report</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>			
Date of submission of the demand  21.12.2005		Date of completion of this report  16.05.2006	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465		Authorized officer  Stoyanov, B  Telephone No. +49 89 2399-7726 	

**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**

International application No.  
PCT/DK2005/000126

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**Box No. I Basis of the report**

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1. With regard to the **language**, this report is based on
- ☒ the international application in the language in which it was filed
  - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
    - ☐ international search (under Rules 12.3(a) and 23.1(b))
    - ☐ publication of the international application (under Rule 12.4(a))
    - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))
2. With regard to the **elements\*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

**Description, Pages**

1-26 as originally filed

**Claims, Numbers**

1-13 received on 29.12.2005 with letter of 21.12.2005

**Drawings, Sheets**

1/2, 2/2 as originally filed

- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
  - ☐ the claims, Nos.
  - ☐ the drawings, sheets/figs
  - ☐ the sequence listing (*specify*):
  - ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
  - ☐ the claims, Nos.
  - ☐ the drawings, sheets/figs
  - ☐ the sequence listing (*specify*):
  - ☐ any table(s) related to sequence listing (*specify*):

\* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**

International application No.  
PCT/DK2005/000126

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**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

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1. Statement

Novelty (N)	Yes: Claims	1-13
	No: Claims	-
Inventive step (IS)	Yes: Claims	1-13
	No: Claims	-
Industrial applicability (IA)	Yes: Claims	1-13
	No: Claims	-

2. Citations and explanations (Rule 70.7):

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
REPORT ON PATENTABILITY  
(SEPARATE SHEET)**

International application No.

**PCT/DK2005/000126**

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**Section V**

The combination of pellet frozen lactic acid bacteria and the additives of claim 1 was not known from the prior art. The present international application provides the unexpected technical effect of increasing the  $T_m$  value of the pellets and keeping them free flowing whilst frozen. Correspondingly, present application is deemed to comply with the requirements of Art. 33(2)(3) PCT.

For the sake of completeness it is noted that present claim 6 has an incorrect dependancy.

It is also noted that present claim 12 is superfluous.

**CLAIMS**

1. A pellet-frozen lactic acid bacteria (LAB) culture in a commercially relevant package that has a weight of at least 50 g frozen material, wherein the frozen material is present in the form of individual pellets, having a content of viable bacteria of at least  $10^9$  colony forming units (CFU) per g frozen material and comprising from 0.5% to 13% of an additive compound measured as w/w of the frozen material, wherein the additive compound is an additive compound that is selected from the group of additive compounds consisting of Cyclodextrin, Maltitol, Trehalose, Fish gelatin, Maltodextrine, Yeast Extract and Spray gum, and which further is characterized by,

when using an amount of 10% of the additive compound measured as w/w of the frozen material, the compound is able to increase the  $T_m'$  (onset temperature of ice melting) of the frozen lactic acid bacteria (LAB) culture, which without the additive compound has a  $T_m'$  value from  $-70^\circ\text{C}$  to  $-46^\circ\text{C}$ , to a  $T_m'$  value above  $-46^\circ\text{C}$ , such as from  $-45^\circ\text{C}$  to  $-15^\circ\text{C}$  (measured by DSC)

and wherein the frozen lactic acid bacteria (LAB) culture is characterized by that when stored at approximately  $-46^\circ\text{C}$  for 7-14 days the individual pellets of the frozen culture are not sticking together and therefore substantially remain as individual pellets where this is measured by following test

the individual pellets of the frozen culture are pellet frozen in liquid nitrogen and 100 individual pellets (around 5 – 100 g of pellets) are poured into a petridish, thus forming a thin layer of loose individual single pellets, the layer being characterized in that the majority of the pellets are in physically contact with one or more of its neighbor pellets, placed at approximately  $-46^\circ\text{C}$  for 7-14 days and examined to see if the pellets are still loose or if the pellets had made clumps or are sticking together wherein the criteria for that the individual pellets of the frozen culture substantially remain as individual pellets are that at least 80 of the 100 individual pellets remain as loose individual single pellets;

with the exception of a frozen lactic acid bacteria (LAB) culture that comprises LAB that are able to utilize sucrose and wherein the culture comprises cryoprotective agent compound selected from the group consisting of sucrose in an amount from 2 % to 13 % of sucrose measured as w/w of the frozen material; and trehalose in an amount from 4 % to 6 % of trehalose measured as w/w of the frozen material; and a trehalose/sucrose mixture both in the amount of 13% measured as w/w of the frozen material.

2. The pellet-frozen culture of claim 1, wherein the culture is a mixed mesophilic culture consisting of mesophilic bacteria having optimum growth temperatures at about 30°C.
3. The pellet-frozen culture of claim 1 or 2, wherein the LAB is a LAB selected from the group comprising *Bifidobacterium* spp., *Brevibacterium* spp., *Propionibacterium* spp., *Lactococcus* spp. including *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*, *Lactobacillus* spp. including *Lactobacillus acidophilus*, *Streptococcus* spp., *Enterococcus* spp., *Pediococcus* spp., *Oenococcus* spp. and fungal spp. including *Penicillium* spp., *Cryptococcus* spp., *Debaryomyces* spp., *Kluyveromyces* spp. and *Saccharomyces* spp.
4. The pellet-frozen culture of any of the preceding claims, wherein the frozen lactic acid bacteria (LAB) culture is a culture which without comprising the additive compound according to claim 1 has a T<sub>m</sub>' value of from -70°C to -46°C.
5. The pellet-frozen culture of any of the preceding claims, wherein the frozen lactic acid bacteria culture comprises from 5% to 10% of the additive compound measured as w/w of the frozen material.
6. A method for making a pellet-frozen lactic acid bacteria (LAB) culture of any of the claims 1 to 6 comprising the following steps:
  - (i) adding an additive compound to viable bacteria to get at least 50 g of material with a content of viable bacteria of at least 10<sup>9</sup> colony forming units (CFU) per g material and comprising the additive compound in an amount from 0.5% to 13% measured as w/w of the material,
  - (ii) freezing the material to get pellet-frozen material, and
  - (iii) packing the pellet-frozen material in a suitable way to get a packed frozen lactic acid bacteria (LAB) culture of any of the claims 1 to 6.

7. The method of claim 6, wherein

before adding the additive compound according to step (i) of claim 6 one has measured the  $T_m'$  value of the frozen lactic acid bacteria (LAB) culture without comprising the additive compound and identified that it has a  $T_m'$  value of from  $-70^{\circ}\text{C}$  to  $-46^{\circ}\text{C}$ ;

and

after adding the additive compound is the  $T_m'$  value of the frozen lactic acid bacteria (LAB) culture comprising the additive compound measured and it is verified that the  $T_m'$  value is from  $-49^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$ , more preferably from  $-39^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$ .

8. The method of claim 6 or 7, wherein the culture is a mixed mesophilic culture consisting of mesophilic bacteria having optimum growth temperatures at about  $30^{\circ}\text{C}$ .

9. The method of claim 6 to 8, wherein the LAB is a LAB selected from the group comprising *Bifidobacterium* spp., *Brevibacterium* spp., *Propionibacterium* spp., *Lactococcus* spp. including *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*, *Lactobacillus* spp. including *Lactobacillus acidophilus*, *Streptococcus* spp., *Enterococcus* spp., *Pediococcus* spp., *Oenococcus* spp. and fungal spp. including *Penicillium* spp., *Cryptococcus* spp., *Debaryomyces* spp., *Kluyveromyces* spp. and *Saccharomyces* spp.

10. The method of claim 6 to 9, wherein the frozen lactic acid bacteria culture comprises from 5% to 10% of the additive compound measured as w/w of the frozen material.

11. The method of claim 6 to 10, wherein the additive compound is an additive compound selected from the group consisting of Cyclodextrin, Maltitol, Trehalose, Fish gelatin, Maltodextrine, Yeast Extract and Spray gum.

12. A pellet-frozen lactic acid bacteria (LAB) culture obtainable by the method for making a frozen lactic acid bacteria (LAB) culture of claim 6 to 11.

13. Use of the pellet-frozen lactic acid bacteria (LAB) culture of any of claims 1-5 and 12 in a process for making a food or feed product.